Long-Term Murine Keratinocyte Cultures Become Tetraploid, Yet Maintain the Ability to Stratify

To the Editor:

We would like to convey a word of caution to readers of the Journal. Two recent reports in this journal describe methods for long-term cultivation of newborn mouse keratinocytes (Hager *et al*, 1999; Caldelari *et al*, 2000). Both reports strongly implied, without disclaimers or caution, that normal mouse keratinocytes could be grown long term from single mouse pups. Hager *et al*, introduce their paper by noting the desirability of culturing mouse keratinocytes from single pups, especially by those who might have generated mice with characterized mutant genes. Indeed, we undertook the use of the Hager method for that purpose and were surprised to find that the cells became polyploid after as few as five passages.

We easily established cell lines from single newborn pups, representing eight different mouse strains and genetically defined mutants. Cells were subcultured every 7–10 d and became homogeneous in appearance and in growth rate, usually after 2 mo in culture or after five to seven passages. After eight to ten passages, five cell lines were tested and found to be nearly tetraploid, as determined by chromosome counts on at least 20 spread mitotic cells (Table I).

All of our lines have the expected characteristics of normal differentiation described by Hager *et al*, and Caldelari *et al*: an epithelial, cobblestone appearance at confluence and expression of biochemical markers of differentiation following elevation of the medium calcium concentration (for example, induction of K1; Fig 1*A*). Moreover, we found that late-passage keratinocytes retain the ability to form a stratified, if somewhat disorganized, epithelium (Fig 1*B*). Some even form a stratum corneum and express late markers of epidermal differentiation, such as keratohyalin granules (Fig 1*Bb*) and immunohistochemical evidence of loricrin expression (not shown).

Table I. Chromosome Analysis of Cultured Mouse Keratinocytes

Strain (transgene)	Chromosomes (weeks *in vitro* at analysis)	Weeks *in vitro* to date
BALB/c	80 ± 2.3 (14)[a]	26
C57BL/6 (SupFG1)	70 ± 16 (9)	33
C57BL/6J (*hprt*$^{-/-}$)	80 ± 3.3 (13)	21
129/SvEvTac (*hfe*$^{-/-}$)	78 ± 4.8 (22)	24
SKH1	77 ± 0.5 (5)	16

[a]Mean ± SD.

It is well known that cultured murine cells are prone to chromosomal instability. With regard to murine keratinocytes, tumorigenic lines spontaneously appear in culture

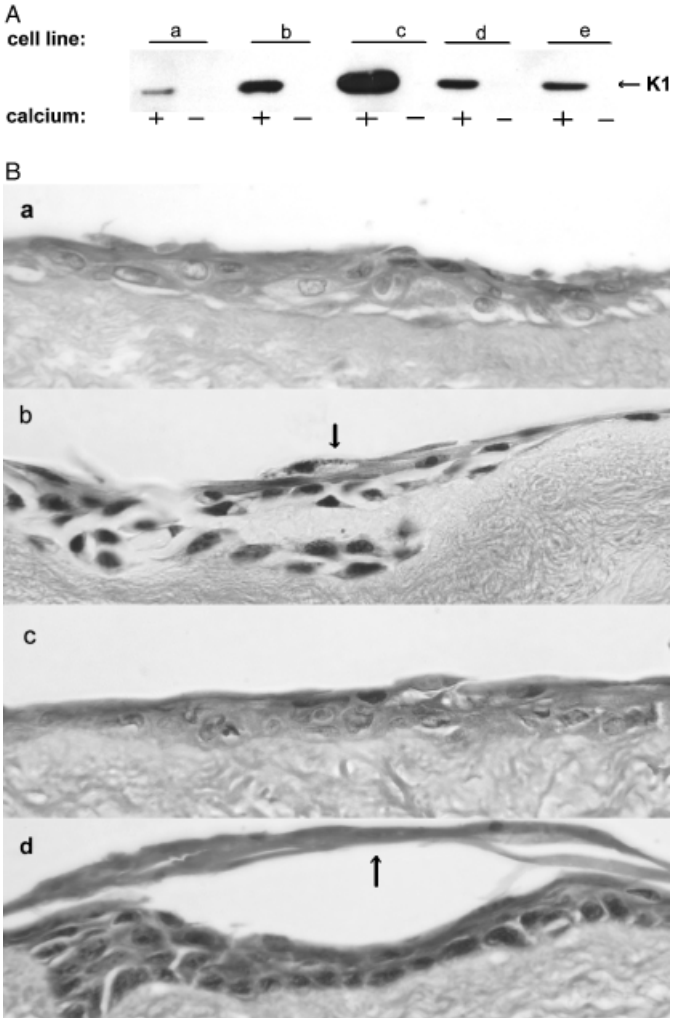

Figure 1 Differentiation and stratification of murine keratinocytes. (*A*) Calcium induction of Keratin 1. Paired samples of cells were grown to confluence in low calcium and then maintained an additional 48 h in either 0.06 mM calcium (−) or 1.8 mM calcium (+). Protein extracts were separated by PAGE and analyzed by western blotting using anti-K1 antibody (Covance, cat. #PRB-165p-100). Cell lines, with passage number in parentheses, are: (*a*) BALB/c (17); (*b*) C57Bl/6^{SupFG1} (20); (*c*) SKH (18); (*d*) 129/SvEvTac$^{hfe-/-}$ (22); (*e*) C57Bl/6$^{hprt-/-}$ (19). (*B*) Keratinocyte stratification. 10^6 cells were seeded onto devitalized dermis (Prunieras *et al*, 1983) and cultured submerged for 1 wk and then at the air:liquid interface for 2 wk. (*a*) C57Bl/6; (*b*) C57Bl/6^{SupFG1}; (*c*) C57Bl/6$^{hprt-/-}$; (*d*) SKH. Arrow in (*b*) indicates keratohyalin granules. Arrow in (*d*) indicates stratum corneum. H&E.

(Yuspa *et al*, 1980), cells exposed to mutagens readily become polyploid (Kulesz-Martin *et al*, 1983), and cells grown in serum-free medium become polyploid after as few as five passages (Kaighn *et al*, 1988). We had hoped that the method of Hager *et al*, might suppress the tendency to polyploidy.

In our current age of expression profiling and searches for quantitative "downstream" effects of defined mutants, long-term murine keratinocyte cultures should be used with caution.

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